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CONCLUSIONS

(1) In complement-fixation tests on 462 sheep of the maternal livestock of one stock farm positive results were obtained in 109 cases (23·6 per cent). In the examination on the same farm of 272 lambs positive results were obtained only in 9 cases (3·3 per cent), the sera of 8 lambs giving a titre of only 8.

(2) Subcutaneous administration of 3 doses of vaccine consisting of a formalinized suspension of *R. burneti* to sheep was accompanied by comparatively rapid antibody formation in high titre, the maximum being observed after 1½–2 months. In 2 control sheep infected intravenously and intratracheally with a large dose of live organisms antibody appeared slowly and reached a maximum only after 5–6 months. The average maximum titre in the vaccinated sheep (before infection) was 2½–3 times as high as in the infected control sheep.

(3) Immediately after infection rickettsias were detected after 24 hr in the blood of 1 vaccinated sheep and also in the faeces of 2 vaccinated sheep (after 24 and 96 hr respectively). In the control animals the organisms were detected in the blood, in 1 after 4 and 7 days and in the other after 7 days. On subsequent periodical investigation of the faeces, urine and milk of the sheep the presence of rickettsias was not observed once over a period of 24 weeks.

(4) The absence of excretion of rickettsias in the faeces, urine and milk of the sheep, together with the extremely slow production of antibody in the control animals evidently indicates the weak reactivity of lambs in relation to *R. burneti*.

(5) In the blood of lambs born to sheep infected with *R. burneti* antibody was found in low titre and disappeared after 2–4 months.

Translated by B. PEARCE

VIRULENCE AND ANTIGENICITY OF A STRAIN OF *BACILLUS ANTHRACIS* CULTIVATED AT 45°—11*

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IN our first communication (Zh. mikrobiol., èpidemiol. immunobiol. No. 1, 1956) we presented data giving evidence of a reduction in the virulence of *Bacillus anthracis* when it is cultivated at a temperature higher than the optimum (45°). The morphological, cultural and biochemical properties of the attenuated strain were not different from those of the initial strain.

In the present paper are set out the results of a study of the virulence and antigenicity of the strain obtained when the conditions of its development are changed. Thus, the virulence of the strain has been tested in laboratory animals after it has been kept for a year in 40% glycerol, subcultured every 24 hr on nutrient media

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and passed through white mice. The purpose of our work was to study more deeply the biological properties of the strain cultivated at 45° and to substantiate this method of attenuating the virulence of *B. anthracis*.

We investigated a culture of the organism obtained by growth in broth for 7 days. Tests in animals showed that a culture containing a concentration of spores not exceeding 60×10^6/ml caused the death of all white mice infected subcutaneously with 0·1 ml, and 1 or 2 of 5 guinea-pigs infected intradermally with 0·2 ml; rabbits infected with 0·5 ml of the culture survived. When there were $77\text{-}80 \times 10^6$ spores per millilitre the culture brought about the death of all the mice and guinea-pigs infected; rabbits survived this dose also. Thus, the culture studied was somewhat more virulent than the first and less virulent than the second of Tsenkovsky's vaccines.

From the basic matrix of the culture mentioned we prepared a sporing form of the culture in 40% glycerol. This culture we passed 41 times in white mice and over a period of 60 days subinoculated it every 24 hr from meat-peptone broth into broth, and from meat-peptone agar on to agar.

The experiments of passing the strains many times through white mice showed that the virulence for mice did not change; the mice through which 0·1 ml of the 24 hr broth culture were passed always died after 35–37 hr. Rare cases of delayed onset of death (up to 47 hr) could be explained by differences in the concentration of organisms in the broth culture used for passage, and also differences in the physiological state of the animals.

The virulence after 10 passages in mice and after 60 subcultures on nutrient media, and also cultures of sporing forms kept for a year in 40% glycerol, were tested in rabbits, guinea-pigs and white mice.

The experiments on white mice (Table 1) showed that a 7 day culture after passage in mice, repeated subinoculation every 24 hr on artificial nutrient media, and also after being kept for a year in 40% glycerol, when injected in a dose of 500,000-7,300,000 spores caused the death of all the animals in 18–56 hr.

The experiments on guinea-pigs established that the strain of *B. anthracis* attenuated by cultivation at 45° stably inherited the altered virulence. The strain passed in mice and on artificial nutrient media, just as before passage caused the death of guinea-pigs in a percentage which varied in accordance with the number of spores injected. Thus, when guinea-pigs were injected with up to 11,200,000 spores all the animals survived, and when they were injected with from 11,600,000 to 15×10^6 spores 1 or 2 out of the 5 animals died. Rabbits survived doses 2½ times as large.

Similar results were also obtained after infection of laboratory animals with a culture which had been kept for 1 year.

The results of these experiments and also observations of the cultural and morphological properties of the given culture provide grounds for concluding that by growing *B. anthracis* in broth at 45° it is possible to obtain a stable variant with the required virulence.

The data presented above allowed us to carry out an experimental investigation of the virulence of the culture under investigation on large agricultural animals, principally on horses. Seventeen horses of average and below-average nutritional state were used in the experiment—work-horses aged from 7 to 18, and also foals of 10-18 months. The horses were clinically healthy, ate normally, drank water and had a normal temperature, the latter being taken morning and evening over a period of several days before the experiment was begun.

All the horses were injected after a 5 day observation period subcutaneously with the culture in spore form in 40% glycerol in saline in the following doses: work-horses 1 ml, foals 0·5–0·7 ml. There were estimated to be 80–85 × 10^6 spores in 1 ml of this culture.

Table 1.

Experimental animals	No. of animals	7 day culture	No. of spores in 1 ml (× 10^6)	Skin dose (in 1 ml)	Results of experiment
White mice (15–18 g)	5	Basic matrix	5–6	0·1	Death after 18–56 hr
	5	Basic matrix	23	0·1	Death after 30–56 hr
	4	Basic matrix	60	0·1	Death after 23–32 hr
	5	Basic matrix one year old	50–58	0·1	Death after 24–36 hr
	5	After 10 passages in white mice	36–56	0·1	Death after 18–56 hr
	5	After 10 passages in white mice	73	0·1	Death after 32–36 hr
	5	After 60 subcultures on media	16–25	0·1	Death after 18–33 hr
Guinea-pigs (400–650 g)	2	Basic matrix	5–6	0·2	Alive
	9	Basic matrix	23–26	0·2	Alive
	3	Basic matrix	60	0·2	One died after 125 hr
	2	Basic matrix	75	0·2	Death after 105–130 hr
	5	Basic matrix one year old	50–58	0·2	One died after 168 hr
	3	After 10 passages in white mice	36–56	0·2	Alive
	5	After 10 passages in white mice	73	0·2	3 died after 108–156 hr
	2	After 60 subcultures on media	16–25	0·2	Alive
Rabbits (1300–1800 g)	2	Basic matrix	5–6	0·5	Alive
	4	Basic matrix	23	0·5	Alive
	2	Basic matrix	26	0·5	Alive
	2	Basic matrix	60	0·5	Alive
	2	Basic matrix	73	0·5	Alive
	2	Basic matrix one year old	50–58	0·5	Alive
	2	After 10 passages in white mice	36–56	0·5	Alive
	2	After 10 passages in white mice	73	0·5	Alive
	2	After 60 subcultures in media	16–25	0·5	Alive

On injection of the culture the animals reacted with a slight rise in temperature (1°–1·5°), there was no oedema at the site of injection, and the general condition of the animals remained completely satisfactory.

From this experiment we drew the conclusion that the culture under investigation is avirulent for horses and could be tried out more widely and on other agricultural animals. To accomplish this task we carried out inoculation of animals, with the approval and agreement of the Ukrainian veterinary administration, on 3 collective farms of the Soldatsk district of Kharkov region, where inoculation against anthrax had not been carried out for a number of years. Horses and cattle were inoculated, including those on individual peasant farms. In all 401 animals were inoculated, of which 248 were horses of various ages and 153 were cattle aged from 1 to 9 years. All these animals were inoculated with the culture intradermally in two places in the region of the neck. The young animals were injected

with 0·5–0·7 ml, the rest with 1 ml. Observation of the condition of the inoculated animals was carried out on the days when we were absent by the local veterinary personnel (a veterinary surgeon and an assistant). A selected group of inoculated animals had their temperatures taken morning and evening.

The temperature 2–3 days after inoculation had risen by 1°–1·5°. At the site of injection some of the horses had swellings the size of a nut, in 2 cases as big as a hen's egg.

It must be mentioned that we did not succeed in establishing a parallel between the dose injected and the extent of the reaction, since in 3 horses to which we intentionally gave a double dose of the culture the reaction did not exceed the usual degree. It seems to us that of fundamental importance for the reaction is the general state of the animals before inoculation, and also the nature of the material used for inoculation.

We tested the antigenicity of the culture in laboratory animals. Guinea-pigs and rabbits were given 1 and 2 doses of culture in sporing form, the guinea-pigs being injected with 0·2 ml and the rabbits with 0·5 ml. In 25–31 days all the animals were infected with a lethal dose of a highly virulent culture of *B. anthracis* titrated in guinea-pigs and rabbits. Of 20 guinea-pigs 13 survived, and 53 of 74 rabbits. The control animals (5 rabbits and 5 guinea-pigs) died of anthrax within 3 days. The results obtained were similar to those obtained from immunization with STI-1 vaccine carried out in 1940–45 by Ginsburg, Spitsin and Tamarin (in their experiments 69–72 per cent of rabbits survived after infection, and 50–60 per cent of guinea-pigs).

Of considerable interest is a group of rabbits given 1 dose of 0·5 ml of the same culture as the animals in the first series of experiments, but after it had been kept for 1 year, and infected intradermally after 30 days with the virulent strain.

TABLE 2. INVESTIGATION OF THE ANTIGENICITY OF A CULTURE OF *B. anthracis* CULTIVATED IN BROTH AT 45° AFTER STORAGE FOR 1 YEAR IN 40% GLYCEROL

Number of rabbits	Dose and method of vaccination	Dose and method of infection	Results of experiment	
			Survived	Died
Experimental 14	0·5 ml intradermally	1 ml of 24 hr broth culture diluted 1 : 1000 intradermally	11 rabbits	3 rabbits in 61–78 hr
Control 2	—	1 ml of 24 hr broth culture diluted 1 : 1000 intradermally	—	2 rabbits in 46–57 hr

The results obtained (Table 2) showed that the animals vaccinated with the attenuated strain which had been kept for 1 year in 40% glycerol acquired immunity to anthrax, and relatively solid immunity was established after a single injection of the culture. Thus, of 14 vaccinated rabbits 11 survived after infection with 5 lethal doses of the virulent strain.

In summary, it can be said that the culture obtained is harmless to rabbits and agricultural animals. On intradermal injection into horses and cattle it did not produce a severe general reaction or extensive oedema at the site of injection, but

it did confer, as can be seen from the results of the experiment with the rabbits, solid immunity against infection.

Thus, as a result of the cultivation of *B. anthracis* in broth at 45° the virulence was reduced but the antigenicity was retained.

CONCLUSIONS

(1) On cultivation of *B. anthracis* at 45° the virulence was reduced to the level of virulence of vaccine strains.

(2) A broth culture of *B. anthracis* cultivated at 45° lay as regards virulence between the first and second of Tsenkovsky's vaccines and possessed high immunizing properties (after a single injection rabbits acquired a high degree of immunity).

(3) On prolonged passage of a 7 day culture on to artificial nutrient media, and also repeated passage in mice, the newly acquired properties were not changed. Therefore this method of attenuation can be used for the purpose of preparing an anthrax vaccine.

Translated by B. PEARCE

VIRULENCE AND ANTIGENICITY OF *SALMONELLA TYPHOSA* STRAINS AND ANTIGENICITY OF THE CORRESPONDING VACCINES IN RELATION TO CONTENT OF VI ANTIGEN AS ESTIMATED BY MEANS OF THE HAEMAGGLUTINATION REACTION*

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EVER since the detection of the surface Vi antigen in *Salmonella typhosa* (Felix, 1934) a great number of studies devoted to this antigen has been published. The relatively complicated methods required, however, caused by the presence of another somatic antigen (O antigen), have made a complete study of the part played by this antigen in the virulence and the antigenicity of the bacteria extremely difficult.

In 1951 Spaun recommended the use of red cell agglutination for the separate study of these 2 antigens. The method is based on the fact that Vi antigen is easily soluble in water and is quickly adsorbed on to red cells (which in consequence become sensitized); the red cells can then be agglutinated by a serum containing Vi antibody, whereas the O antigen remains fixed to the bacterial cell. These findings were confirmed by Courvoisier (1952), Minor, Minor and Grabar (1952),

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